HISTIDINE PROTEIN PHOSPHATASE

The invention relates to a novel enzyme, histidine protein phosphatase, which is derived from mammalian sources, and its homologue variants. The invention further relates to DNA sequences encoding said proteins, to a process for preparing the latter, and to antibodies directed against them. The novel phosphatase can be used for diagnosis of pathological states of cell regulation and cell growth and as pharmaceutical drug which can be administered in conjunction with pathological disorders related to malfunctions of said enzyme. In detail this invention relates to the application of said enzyme in diagnosis, treatment of disease, agonists and antagonists in identifying factors that function in histidine phosphorylation, as well as agonists and antagonists that are potentially useful in therapy.

Background of the Invention

Protein phosphorylation is a fundamental biological process that often plays a key role in signal transduction and in the regulation of protein activity. Phosphorylation of serine, threonine, and tyrosine is common in eukaryotic signal transduction pathways. There is an extensive literature on biochemical methods for detection of phosphorylation of serine, threonine, and tyrosine on unknown proteins. For example, when mammalian cultured cells are treated with growth factors, cytokines, drugs, peptides, or other stimulatory molecules such as PDGF, NGF, IL-2, etc., phosphorylation of proteins at serine, threonine, and tyrosine can easily be detected by incubating the cells with radiolabeled phosphate, isolating proteins after treatment with a growth factor, etc., separating the proteins by SDS polyacrylamide gel electrophoresis, fixing the gel, and performing autoradiography to identify protein bands containing the labeled phosphate. Alternatively, proteins from treated and untreated cells can be separated using a two-dimensional system, and the position of protein spots can be compared. The apparent movement of a spot can indicate a change in modification state that results from a treatment. In another method, proteins are subject to acid hydrolysis to yield amino acids that can then be subjected to thin-layer chromatography and examined for the presence of radiolabeled phosphorus.

However, these protein chemistry methods employ conditions in which phosphorylated histidine, lysine, or arginine is unstable. In particular, phosphohistidine spontaneously breaks down with a half-life of about 5 – 100 minutes at low pH, but at neutral pH phosphohistidine has a half-life of days to weeks (Matthews, H. R. [1995] Pharmac. Ther. 67:323-350). For example, following electrophoresis, polyacrylamide gels for protein separation are generally fixed under acidic conditions. Similarly, the acidic conditions for amino acid hydrolysis also lead to breakdown of phosphohistidine.

Phosphorylated histidine has been demonstrated to play a key role in signal transduction in bacteria. Such pathways regulate many aspects of bacterial metabolism. For example, the ArcA/ArcB system governs aerobic and anaerobic metabolism in E. coli (Iuchi S, Weiner L. J Biochem (Tokyo) [1996] 120:1055-63). OmpR and OmpF govern the response to different osmotic conditions (Pratt LA, Hsing W, Gibson KE, Silhavy TJ. Mol Microbiol [1996] 20:911-7). CheA and CheZ are involved in chemotaxis (Alon U. Nature [1999] 397:168-171).
All of these proteins were first identified by genetic means: mutations in the corresponding genes cause phenotypes of interest, and the resulting proteins can be identified after cloning the relevant genes.

Similarly, it has recently been found that the eukaryotes *S. cerevisiae* and *Arabidopsis thaliana* have signal transduction systems with proteins that involve phosphorylation of histidine (Loomis et al., [1997] J. Cell Science 110:1141-1145). For example, the Sln1 protein has an extracellular sensor domain, a cytoplasmic histidine kinase domain, and an aspartate relay domain. ETR1 of the mustard plant *Arabidopsis thaliana* was also identified by mutants that fail to respond to ethylene. The ETR1 gene was then cloned and the protein studied, leading to the discovery that this protein is a histidine kinase. *S. cerevisiae* and *Arabidopsis thaliana* are genetically tractable organisms, and their proteins involved in histidine phosphorylation, such as Sln1 and Etr1, are usually identified by genetic means.
Existing techniques generally involve size separation of either proteins or phosphoamino acids.

Mammalian N-phosphorylation is important in energy metabolism and is altered in various types of cancer. For example, the Nm23 protein is a nucleoside diphosphate kinase that can use the ATP generated by glycolysis and oxidative phosphorylation to convert nucleoside and deoxynucleoside diphosphates into triphosphates. Nm23 becomes transiently phosphorylated on Histidine 118 as part of its ping-pong reaction mechanism. Nm23 is also capable of transferring its phosphate group onto histidine residues in ATP-citrate lyase and succinyl CoA synthetase. Thus, histidine phosphorylation plays an important role in energy metabolism in cells.

In humans there are several Nm23 proteins. In highly metastatic cancers, these Nm23 proteins are often expressed at low levels. Based on these results, Nm23 is thought to be an anti-oncogene. In addition, in certain cancers, mutations in particular Nm23 proteins are found. These mutations often affect the rate of phosphorylation or dephosphorylation of Nm23.

Taken together, these results indicate that N-phosphorylation plays an important role in energy metabolism and in cancer. Thus, proteins or drugs that modulate N-phosphorylation or dephosphorylation could be important in treatment of cancer or metabolic disorders such as obesity, anorexia, wasting due to cancer, HIV or other diseases, and so on. In addition, since N-phosphorylation plays a role in a wide variety of biological phenomena in other organisms, it may be that N-phosphorylation plays a role in other mammalian diseases and disorders, such as immune disorders, viral infection, genetic disorders, heart disease, and so on.

Unfortunately, mammals grow much more slowly than bacteria, *S. cerevisiae* and *Arabidopsis thaliana*, and it is therefore difficult to identify proteins involved in histidine, lysine, or arginine phosphorylation by the same genetic approaches used for these simpler organisms. In addition, biochemical techniques for identifying proteins that are phosphorylated on serine, threonine, and tyrosine are not generally applicable to identifying proteins that are phosphorylated on histidine, lysine, and arginine. There is therefore a need in the art for techniques and biochemical reagents for use in studying N-phosphorylation.